435/69.1 print page

Exo III Generated Structures

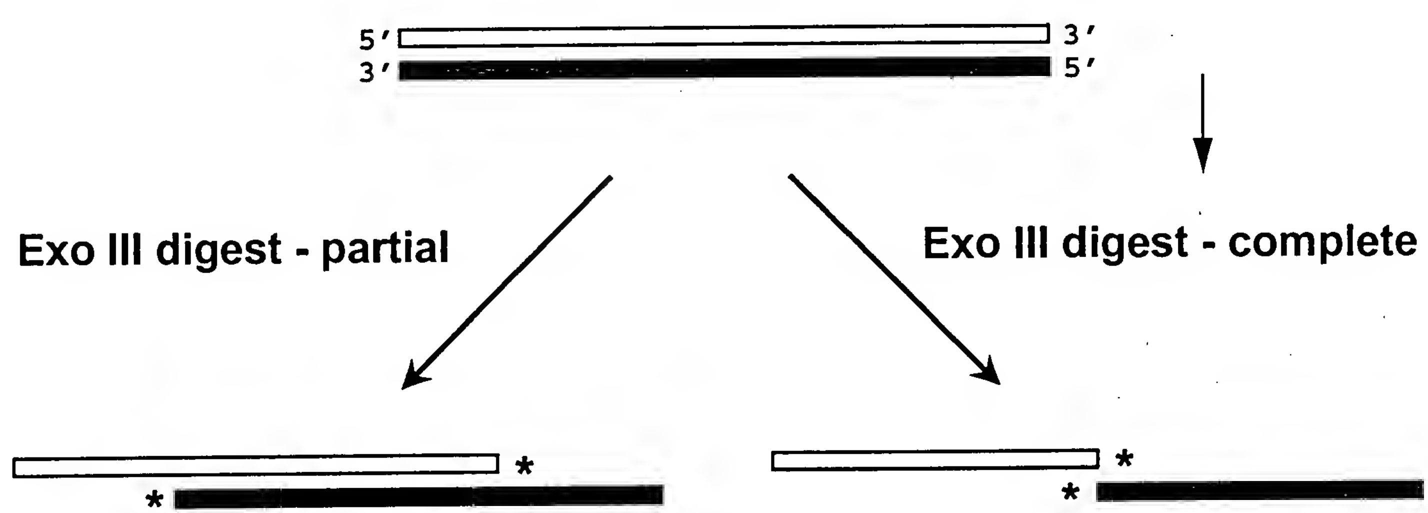


Figure 1

Figure 2. Generation of A Nucleic Acid Building Block by Polymerase-Based Amplification.

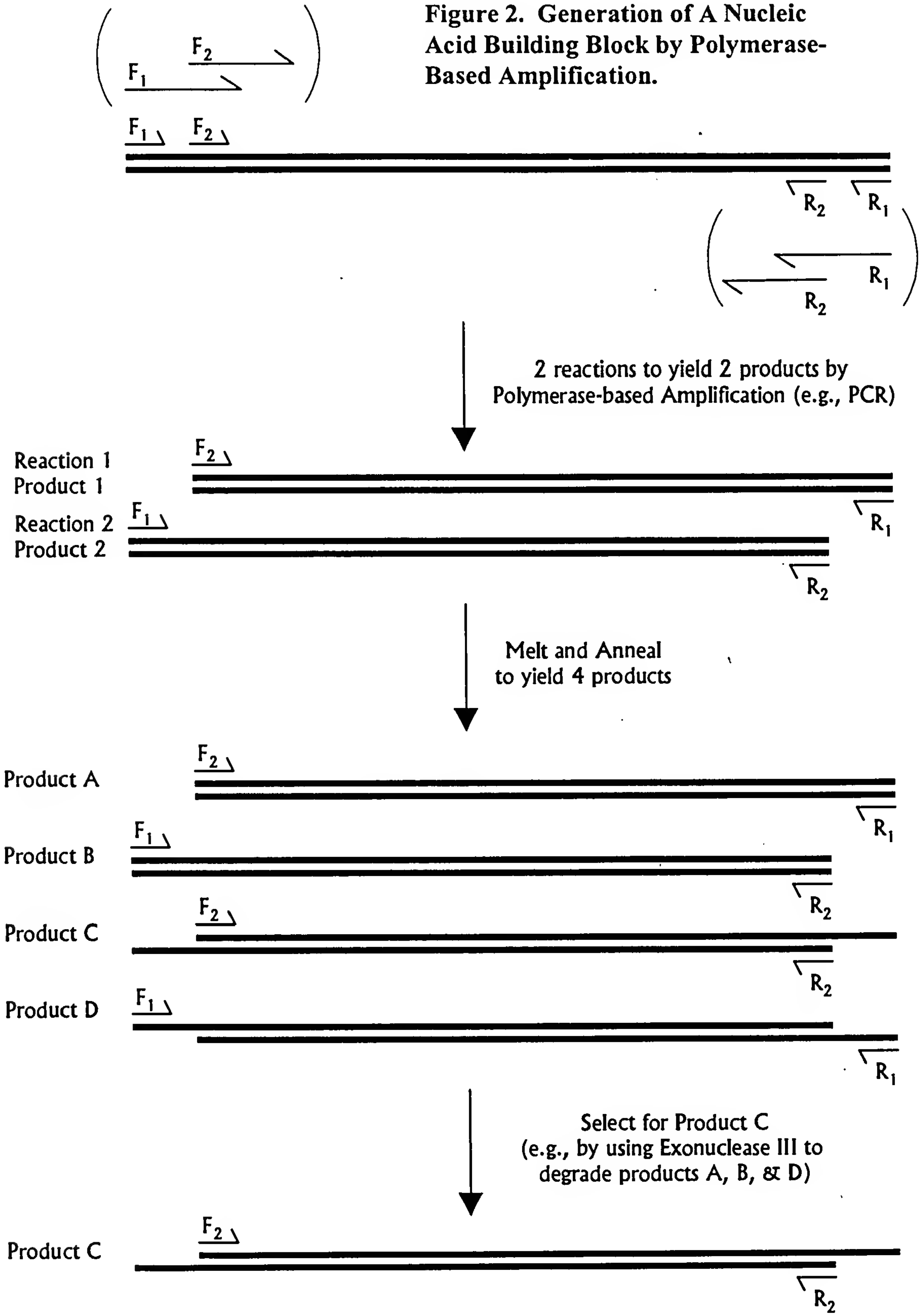
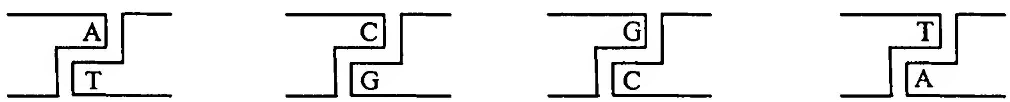


FIGURE 3. Unique Overhangs And Unique Couplings.

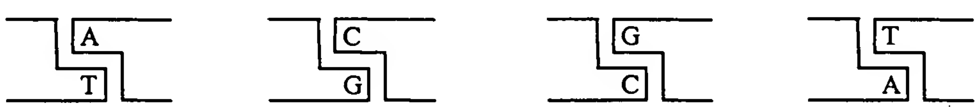
The number of unique overhangs of each size (e.g. the total number of unique overhangs composed of 1 or 2 or 3, etc. nucleotides) exceeds the number of unique couplings that can result from the use of all the unique overhangs of that size. For example, the total number of unique couplings that can be made using all the 8 unique single-nucleotide 3' overhangs and single-nucleotide 5' overhangs is 4.

PANEL A. 4 unique single-nucleotide 3' overhangs are possible (i.e., A, C, G, & T). For each of these there is a complementary 3' overhang with which it can pair (i.e., T, G, C, & A, respectively), as shown.



PANEL B. However, the number of unique single-nucleotide 3' overhangs is greater than the number of unique couplings. Thus, only 2 intrinsically unique couplings exist using single-nucleotide 3' overhangs as shown.

PANEL C. Likewise, 4 unique-single nucleotide 5' overhangs are possible (i.e., A, C, G, & T). For each of these there is a complementary 5' overhang with which it can pair (i.e., T, G, C, & A, respectively), as shown.

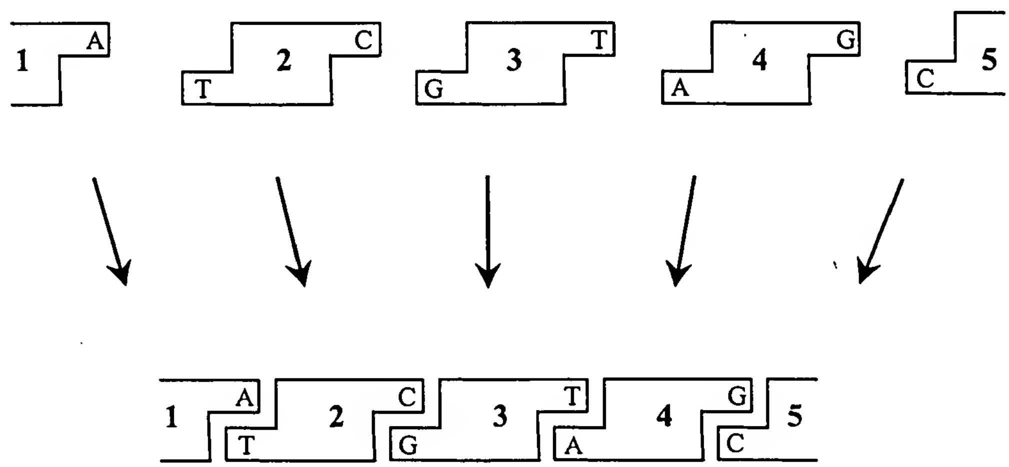


PANEL D. However, the number of unique single-nucleotide 5' overhangs is greater than the number of unique couplings. Thus, only 2 intrinsically unique couplings exist using single-nucleotide 5' overhangs as shown.

FIGURE 4. Unique Overall Assembly Order Achieved by Sequentially Coupling the Building Blocks

Awareness of the degeneracy (between the number of unique overhangs and the number of unique couplings) is important in order to avoid the production of degeneracy in the overall assembly order of the finalized nucleic acid. However, a unique overall assembly order can also be achieved - despite the use of non-unique couplings - by using building blocks having distinct combinations of couplings, and/or by stepping the assembly of the building blocks in a deliberately chosen sequence.

PANEL A. For example, one could attempt to assemble the following nucleic acid product using the 5 nucleic acid building blocks as shown.



PANEL B. However, degeneracy in the overall assembly order of the 5 nucleic acid building blocks would be present if the assembly process were carried out in one step. For example, building block #2 and building block #3 could both couple to building block #1 as shown.

Figure 5. Unique Couplings Available Using a Two-Nucleotide 3' Overhang.

16 unique 3' overhangs can be formed using two nucleotides. However, use of these 16 unique overhangs allows for the formation of only 6 unique couplings. Another 6 unique couplings are provided by the use 5' overhangs formed using two nucleotides. Thus, a total of 12 unique couplings are provided by the combined use of 3' and 5' two-nucleotide overhangs. "Twin" couplings are marked in the same shading.

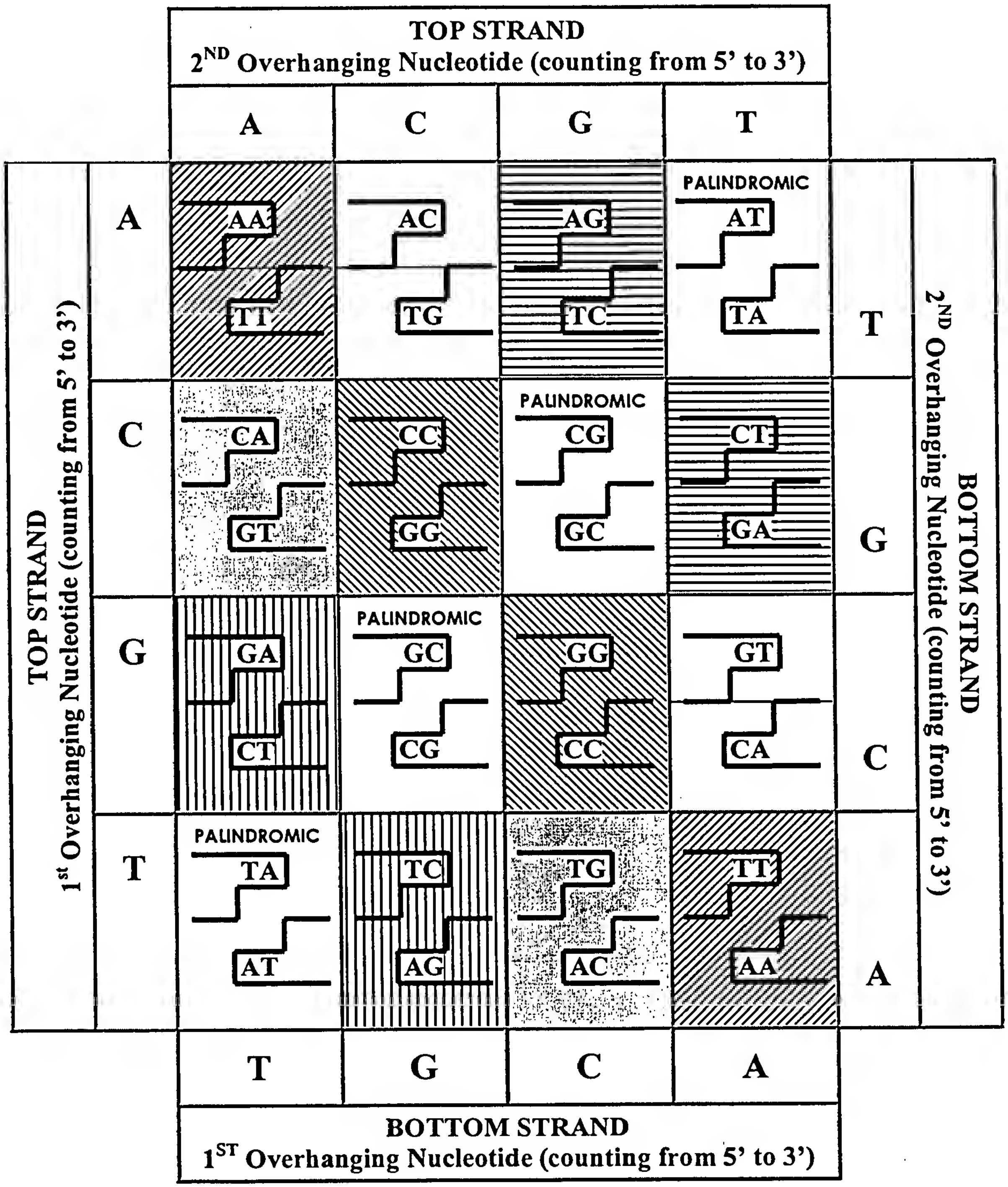


FIGURE 4 cont.

PANEL C. However, a unique overall assembly order could be achieved by sequentially coupling the building blocks in 2 steps (rather than all at once) as shown.

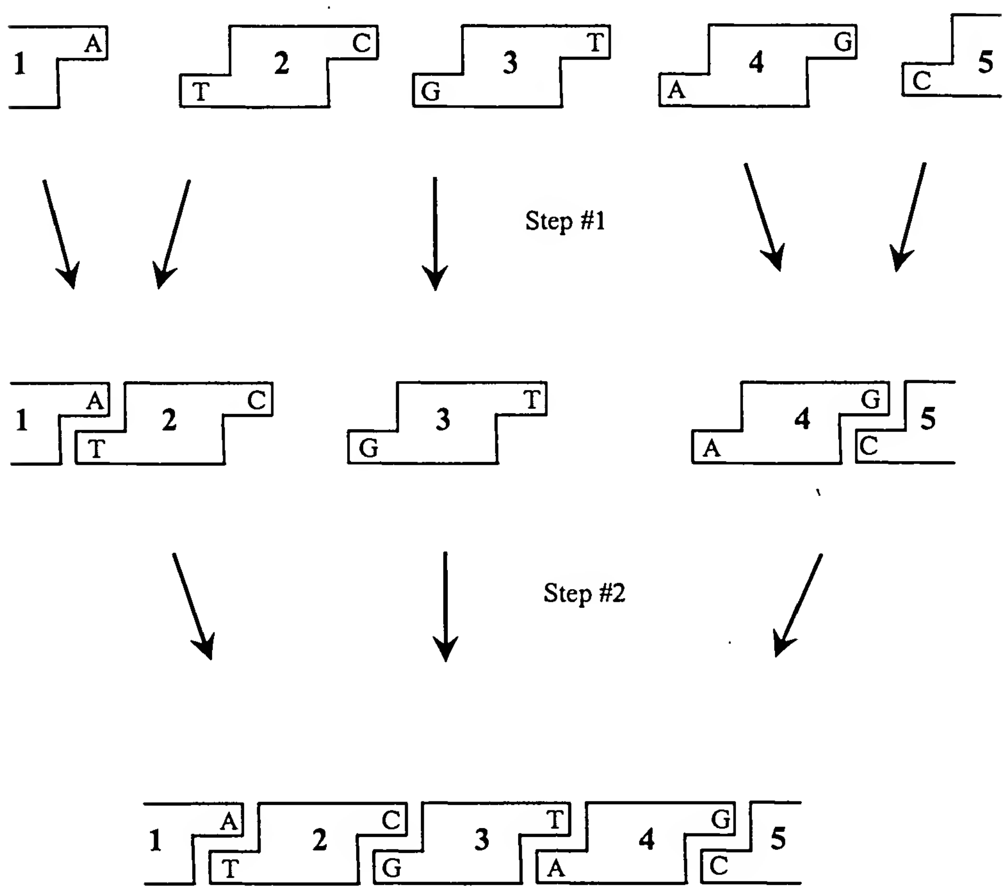
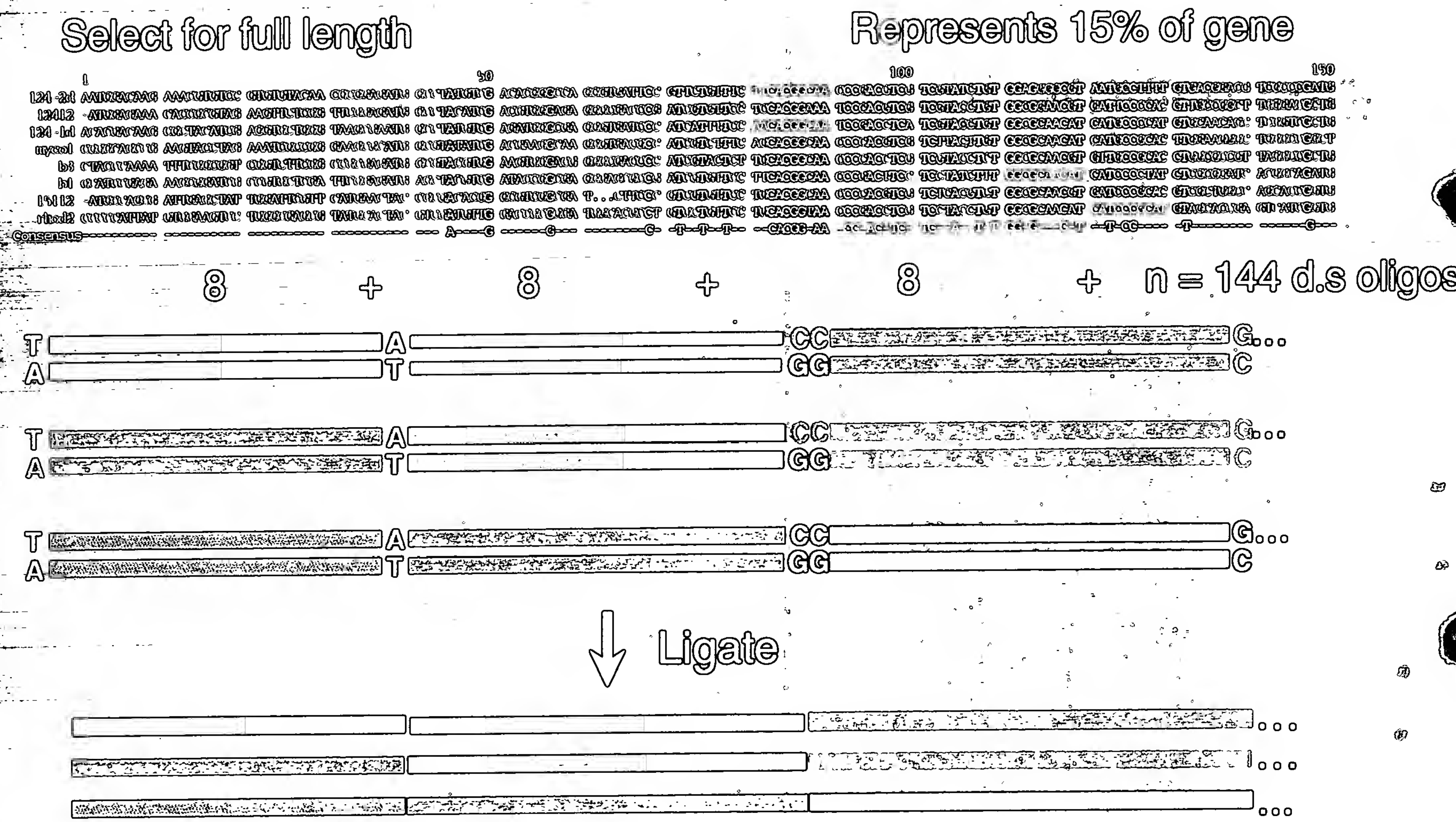


Figure 6. Generation of an Exhaustive Set of Chimeric Combinations by Synthetic Ligation Reassembly.



$8^{18} = 2x10^{16}$ Reassembled Gene Variants

Figure 7. Synthetic genes from oligos.

```
             NcoI                                                          CCGT
150am13_00   c ATGATGCACG GCGATATTTC ATCGAGCAAT GACACGGTCG GCGTTGCCGT
150AM7_001   c ATGCATCACG GCGACATTTC ATCGAGCAAT GACACGGTCG GCGTTGCCGT
431am7_002   c ATGAGACACG GAGATATCTC CAGCAGCAAC GATTGCGTGG GCGTGGCCGT

                                                           GAG  GT
150am13_00     CGTGAACTAC AAGATGCCTC GCCTTCATAC CAAGGCGGAG GTTTTAGCGA
150AM7_001     CGTGAACTAC AAGATGCCGC GGCTTCACAC CAAGGCTGAG GTGCTGGCCA
431am7_002     CGTGAACTAC AAGATGCCGC GGCTGCATAC CCGCGCGGAG GTGATGGAGA

                                                        CGG
150am13_00     ACGCCAGAAA GATCGGCGAG ATGATCGTCG GCATGAAGAC CGGCCTGCCC
150AM7_001     ACTGCCGCAA GATCGCCGAC ATGCTGGTCG GCATGAAGAG CGGCCTGCCG
431am7_002     ACGCCCGCAA GATCGCCGAC ATGGTCGTGG GCATGAAGCG CGGCCTGCCC

                                                  CCACG
150am13_00     GGAATGGATC TGGTGATCTT CCCGGAATAT TCGACCCACG GCATCATGTA
150AM7_001     GGAATGGATC TGGTGATCTT CCCGGAATAT TCCACCCACG GCATCATGTA
431am7_002     GGCATGGACC TGGTCATCTT CCCCGAGTAC TCCACCCACG GCATCATGTA

                                                        CCC  GG
150am13_00     CGACTCCAAG GAAATGTACG ATACCGCGTC CGTCGTGCCC GGCGAGGAGA
150AM7_001     CGACTCCAAG GAGATGTACG ACACGGCGTC GACGGTGCCG GGTGAAGAGA
431am7_002     CGACGCCAAG GAAATGTACG AAACCGCTTC GGCCATTCCG GGCGAAGAGA

                                                        G  GGG
150am13_00     CCGAGATTTT TGCCGAAGCC TGCCGCAAGG CGAAAGTCTG GGGCGTGTTC
150AM7_001     CCGAGATTTT CGCCGAGGCC TGCCGCAAGG CCAAGGTCTG GGGCGTGTTC
431am7_002     CTGCTGTGTT CGCCGACGCC TGCCGCAAGG CCAACGTATG GGGCGTGTTT

                                                   AAAG  C
150am13_00     TCGCTCACCG GCGAACGTCA CGAGGAACAT CCGAAGAAGG CGCCCTACAA
150AM7_001     TCGCTGACCG GCGAGCGCCA CGAGGAGCAT CCCAATAAAG CGCCGTACAA
431am7_002     TCGCTGACGG GCGAGCGCCA CGAAGAGCAC CCGAACAAGG CGCCGTACAA

                                                     CAG  AA
150am13_00     CACGCTGATC CTGATGAACG ACAAGGGCGA GGTGGTCCAG AAATACCGCA
150AM7_001     CACCCTGATC CTGATGAACG ACAAGGGTGA AGTCGTTCAG AAATATCGCA
431am7_002     CACGCTCATC CTGATGAACA ACAAGGGCGA GATCGTGCAG AAGTACCGCA

                                              GGTA
150am13_00     AGATCATGCC GTGGGTTCCG ATCGAGGGCT GGTACCCCGG CAACTGCACC
150AM7_001     AGATCATGCC GTGGGTGCCG ATCGAAGGCT GGTATCCCGG CAACTGCACG
431am7_002     AGATCATGCC CTGGGTGCCG ATCGAAGGCT GGTATCCGGG CGATTGCACC

                                       TGAAG
150am13_00     TACGTCTCCG ACGGGCCGAA GGGCATGAAG GTTTCGCTGA TCATCTGCGA
150AM7_001     TACGTCTCCG AAGGCCCGAA GGGCATGAAG ATGTCGCTGA TCATCTGCGA
431am7_002     TATGTGTCGG AAGGCCCCAA GGGACTGAAG ATCAGCCTCA TCATCTGCGA

                                  TCTGGCG
150am13_00     TGACGGCAAC TATCCGGAAA TCTGGCGCGA CTGCGCCATG AAGGGCGCCG
150AM7_001     CGACGGCAAC TACCCGGAAA TCTGGCGTGA CTGCGCGATG AAGGGCGCCG
431am7_002     CGACGGCAAT TACCCCGAGA TCTGGCGCGA TTGCGCCATG CGCGGCGCCG
```

Figure 7 cont.

```
                             CCAG
150am13_00    AGCTGATCGT GCGCTGCCAG GGCTACATGT ATCCGGCCAA GGACCAGCAG
150AM7_001    AACTGATCAT CCGCTGCCAG GGCTACATGT ATCCCGCCAA GGATCAGCAG
431am7_002    AGCTGATCGT GCGTTGCCAG GGATACATGT ACCCGGCCAA GGACCAGCAG

                           GC
150am13_00    GTCATCATGG CGAAGGCGAT GGCGTGGGCG AATAATTGTT ACGTCGCGGT
150AM7_001    GTGCTGATGG CGAAAGCAAT GGCCTGGGCC AACAACGTTT ATGTCGCGGT
431am7_002    GTCATGGTGT CCAAGGCCAT GGCGTGGATG AACAACGTCT ACGTGGCGGT

                        GGGCTTCG
150am13_00    TTCCAATGCC GCGGGCTTCG ATGGCGTCTA TTCGTATTTC GGCCACTCGG
150AM7_001    CGCCAATGCC TCGGGCTTCG ACGGCGTCTA CTCGTATTTC GGCCATTCGG
431am7_002    GGCCAATGCC GCGGGCTTCG ACGGCGTGTA TTCCTACTTC GGCCATTCGG

                        TTCGA
150am13_00    CGATCATCGG CTTCGATGGC CGCACGCTCG GCGAATGCGG CGAGGAAGAA
150AM7_001    CGATCATCGG CTTCGACGGC CGTACCCTCG GCGAATGCGG CGAGGAGGAT
431am7_002    CCATCATCGG CTTCGACGGC CGCACGCTGG GCGAATGCGG TGAAGAAGAC

                       C AGTA
150am13_00    TACGGCATCC AGTATGCCCA GCTTTCGAAG ATGCTGATCC GCGACGCCCG
150AM7_001    TATGGCATCC AGTATGCCGC CATCTCCAAG TCGCTGATCC GCGACGCGCG
431am7_002    ATGGGCGTGC AGTACGCCGA GCTCTCCACC AGCCTGATCC GCGACGCGCG

                        CAATC
150am13_00    CCGCACCGGA CAATCGGAAA ACCATCTCTT CAAGCTGGTG CATCGTGGCT
150AM7_001    CCGCACCGGC CAATCGGAAA ACCATCTCTT CAAGCTGGTG CACCGTGGCT
431am7_002    CAAGAACATG CAGTCGCAGA ACCACTTGTT CAAGCTGGTG CACCGCGGCT

                        GATCAA
150am13_00    ACACCGGGTT GATCAACTCC GGCGAGGGCG ACCGCGGTCT CGCGGCCTGT
150AM7_001    ACACCGGCAT GATCAATTCC GGCGAGGGCG ACCGCGGTGT CGCGGCTTGC
431am7_002    ACACCGGCAA GATCAATTCC GGCGAAGAGG CCACCGGCGT CGCGGCATGC

                TTA
150am13_00    CCTTATGAGT TCTACAACAA ATGGATCGCC GATCCGGAAG GCACCCGCGA
150AM7_001    CCGTATGATT TCTATTCGAA ATGGATCGCC GATCCCGAGG GTACACGCGA
431am7_002    CCGTACAACT TCTACGCCAA CTGGATCAAC GATCCGGAGG GCACGCGCAA

               ATGGT
150am13_00    AATGGTCGAG TCCTTTACCC GGCCGACGGT GGGAACCGAT GAAGCGCCCA
150AM7_001    GATGGTGGAA TCCTTCACGC GTCCGACGGT GGGTGTGGAG GAATGCCCGA
431am7_002    GATGGTCGAA TCCTTCACCC GGTCCACCGT GGGCACGCCG GAGTGCCCCA

              TCGAG
150am13_00    TCGAAGGCAT CCCGAACAAG GTCGCGGTGC ACCGCTGA    aagct
150AM7_001    TCGAGGGCAT TCCGAACAAG GCCACCACGC ACCGCTGA    aagct
431am7_002    TGGACGGCAT CCCCAACGAG GACGCCAAGC ACCGCTAG    aagct
                                                            HindIII
```

Figure 8. Nucleic acid building blocks for synthetic ligation gene reassembly.

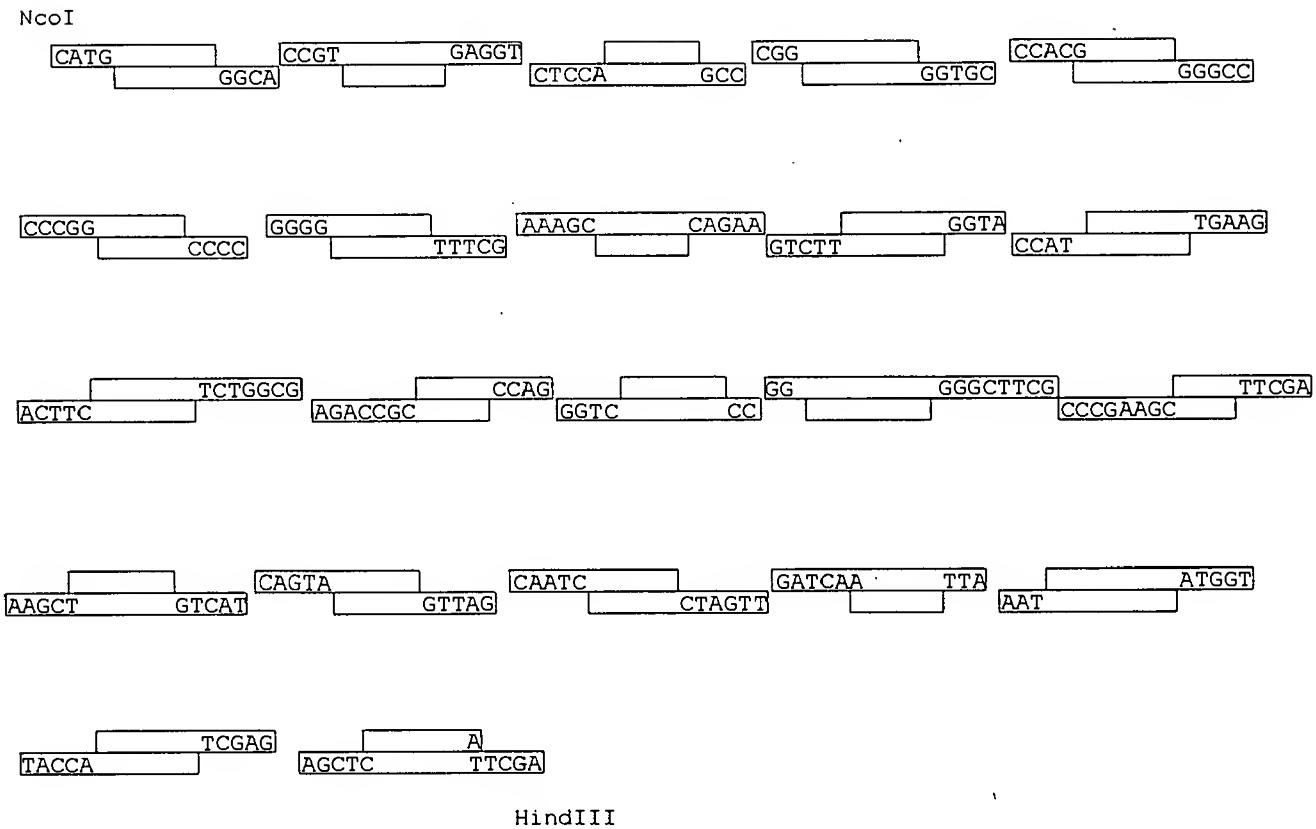
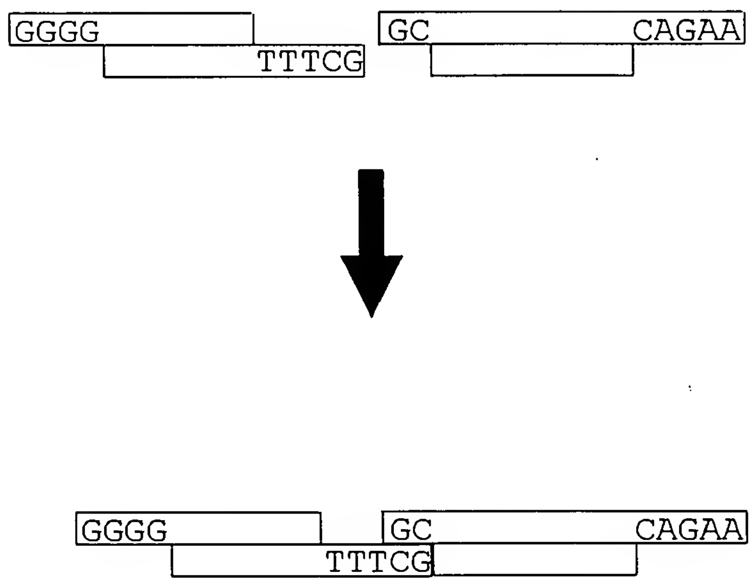


Figure 9. Addition of Introns by Synthetic Ligation Reassembly.

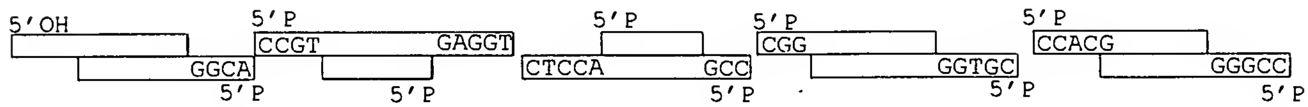
Figure 10. Ligation Reassembly Using Fewer Than All The Nucleotides Of An Overhang.

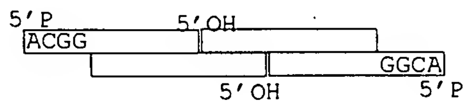
Gap Ligation



Ligation of one strand only;
gap in second strand can be repaired in vivo

Figure 11. Avoidance of unwanted self-ligation in palindromic couplings.





No self ligation of end primers
with palindromic overhangs

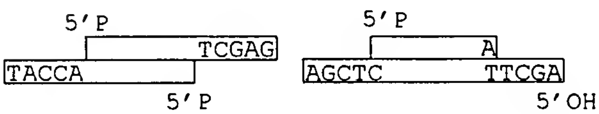


Figure 12. Pathway Engineering

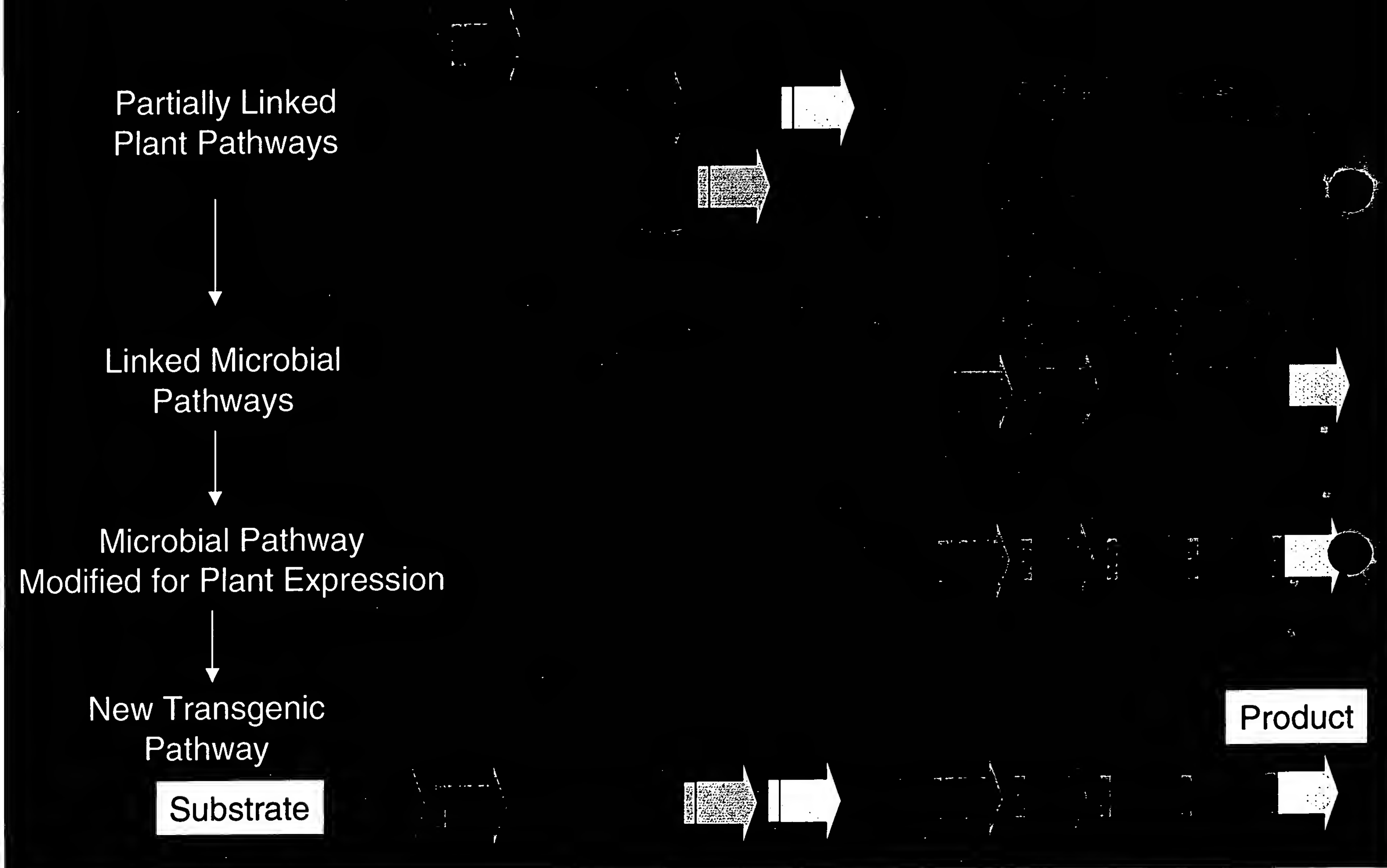


Figure 13. Pathway Improvement / Evolution

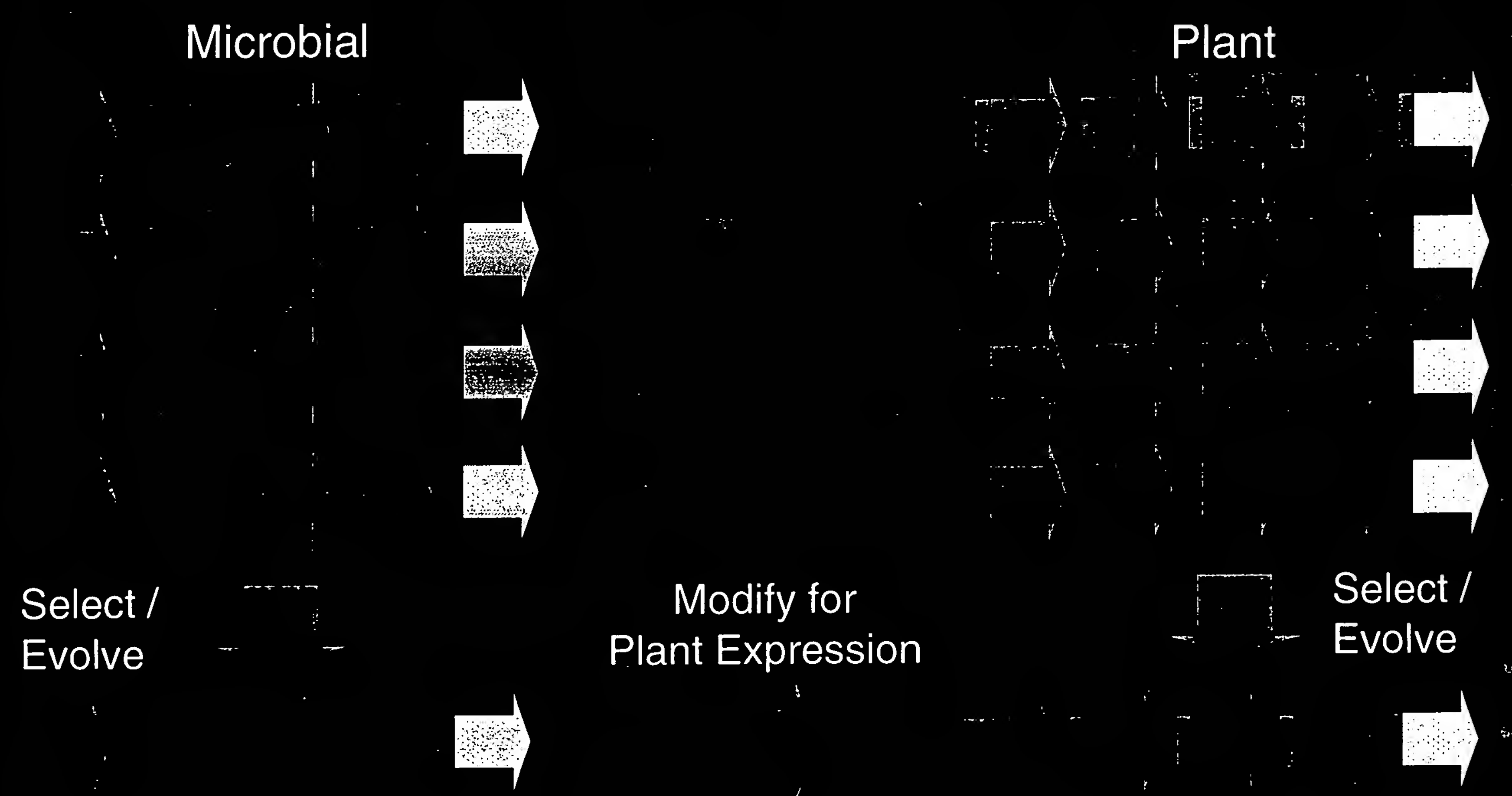


Fig 14. Conversion of Microbial Pathways to Eukaryotic Pathways

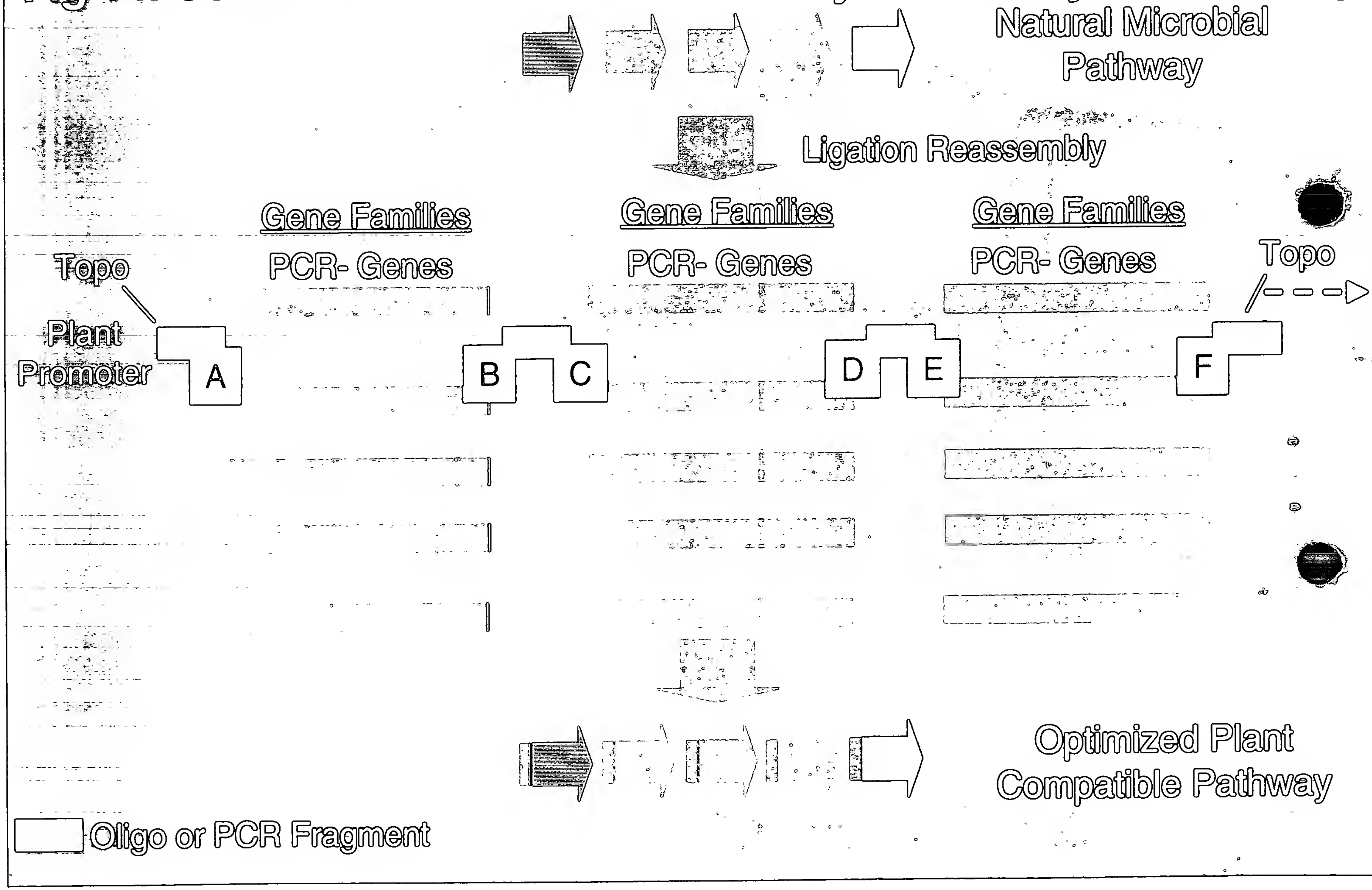


Figure 15

Evolution of polypeptides by synthesizing (in vivo or in vitro) corresponding deduced polynucleotides and subjecting the deduced polynucleotides to directed evolution and expression screening subsequently expressed polypeptides.

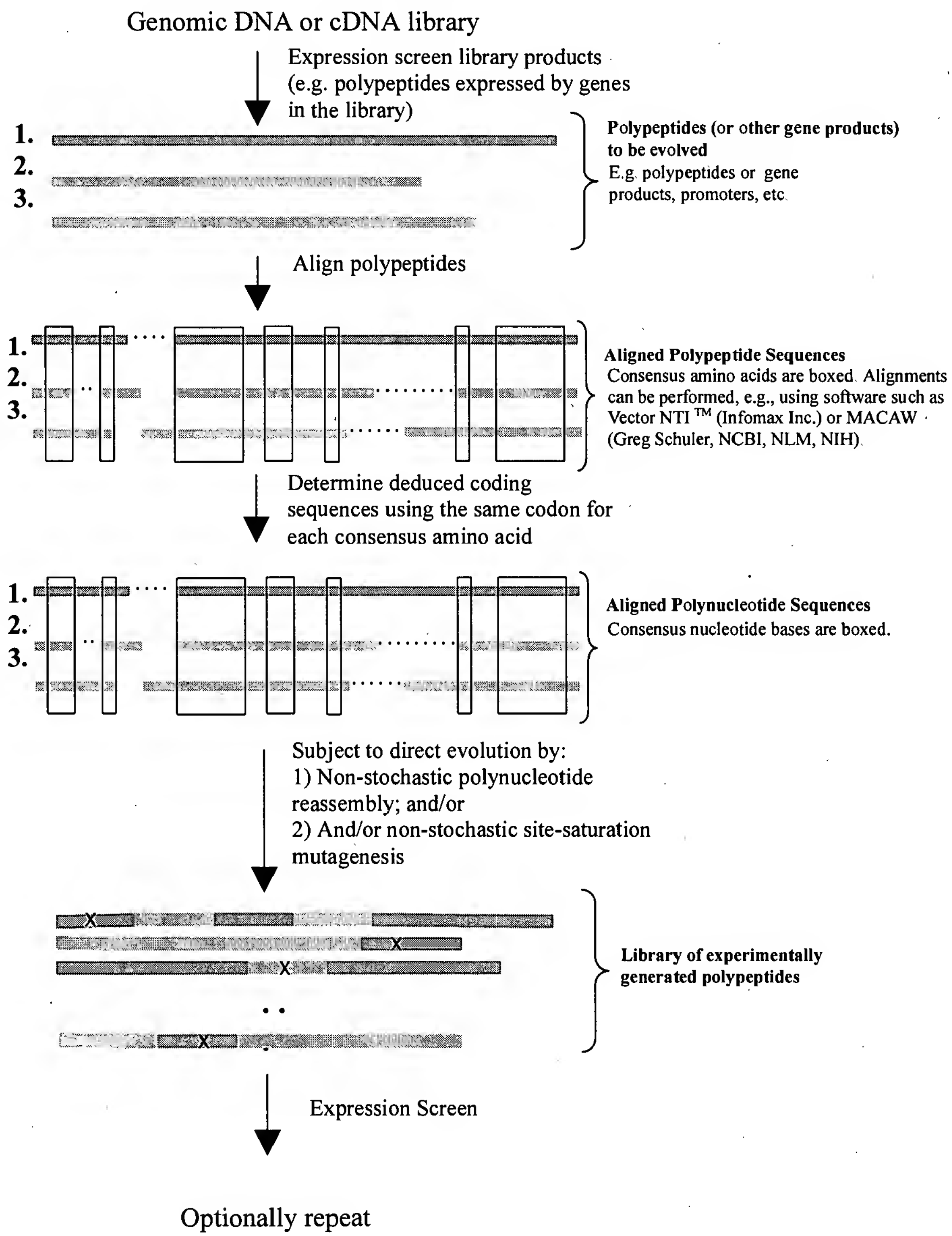
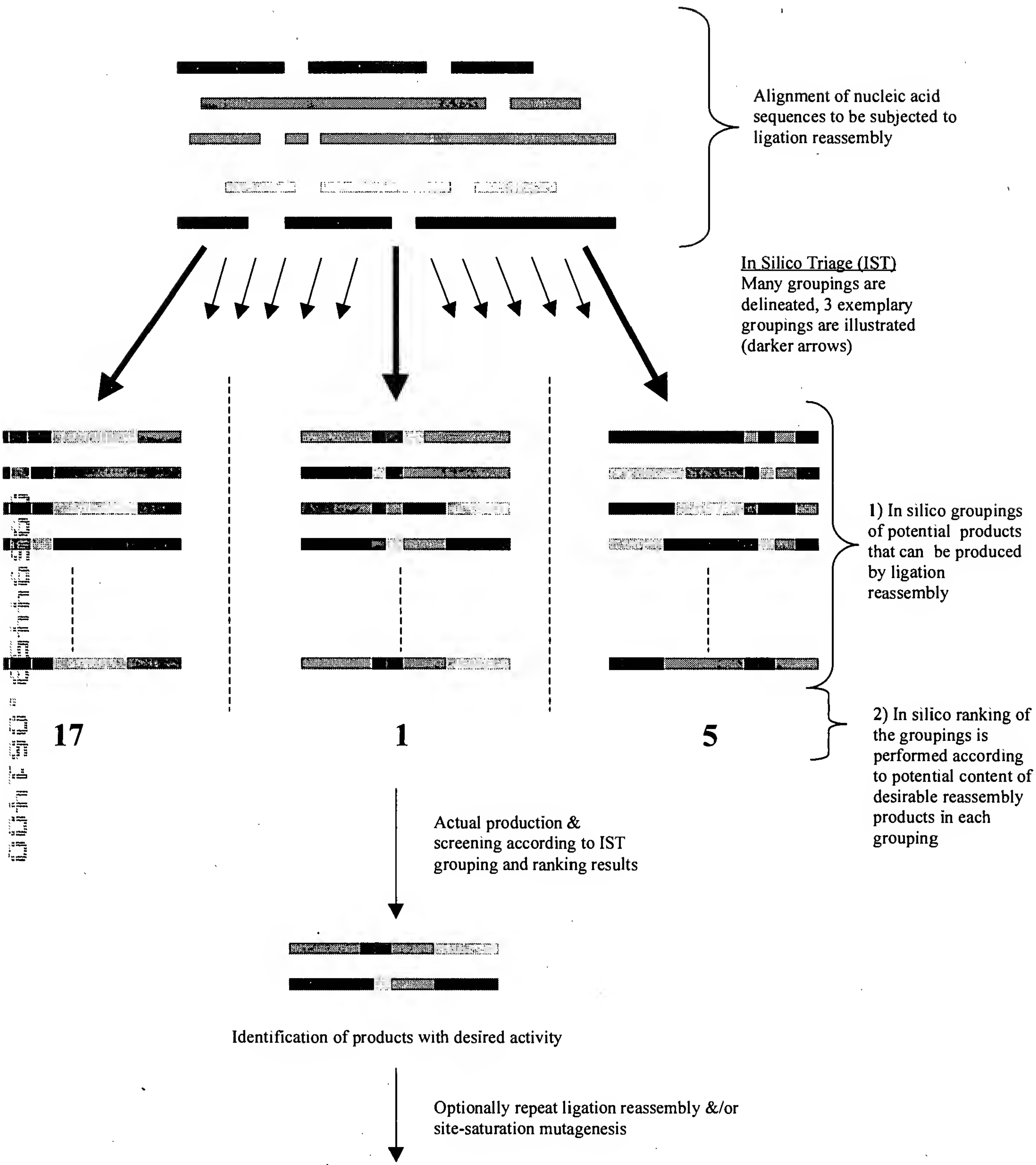
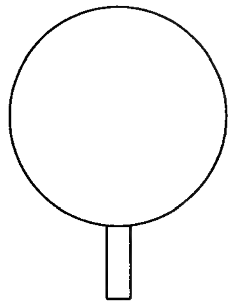


Fig. 16: In Silico Triage





Streptavidin-conjugated
Magnetic beads

```
                                                F1                    F2                         F3                     F4
                                                85-bp                 49-bp                      36-bp                  48-bp
5'Biotinyl-spacer-TOPO sequence-AAGGTATTG--------GGCCTTC CGGACCGC----------TTCATCGAG CAGGTGCT-------CCGCTTACG GAGGTCGAG--------
                                     ATAAC--------CCGGAAGGCC TGGCG----------AAGTAGC TCGTCCACGA-------GGCGAATG CCTCCAGATC--------
GGACAACT

                                F5
                                45-bp
   ---CCTGTTGACCGA GAGCCACTG--------GCCGGTGAG
                 GGCTCTCGGTGAC--------CGGCCACTCGGGC
```

Outline of the procedure

1. Annealing of complementary oligos.
   (Couplings are shown underlined & in bold. Dashes "------" indicate internal sequences not involved in couplings.
2. Immobililization of 5' pre-annealed biotinytated fragment to conjugated beads.
3. Wash to remove free fragments.
4. Enzymatic ligation of consecutive pre-annealed fragments including washes between each addition to remove free fragments.
5. BsaI-mediated elution of reassembled gene (cuts inside the TOPO sequence).
6. Ligation to 5'- and 3'end PCR generated fragments (if necessary).
7. Cloning into appropriate vector.

Fig. 17: Solid Phase Ligation Reassembly

Figure 18

Non-stochastic polynucleotide reassembly in combination with non-stochastic polynucleotide site-saturation mutagenesis.

Shown below is a non-limiting example of a permutation of the directed evolution methods described herein

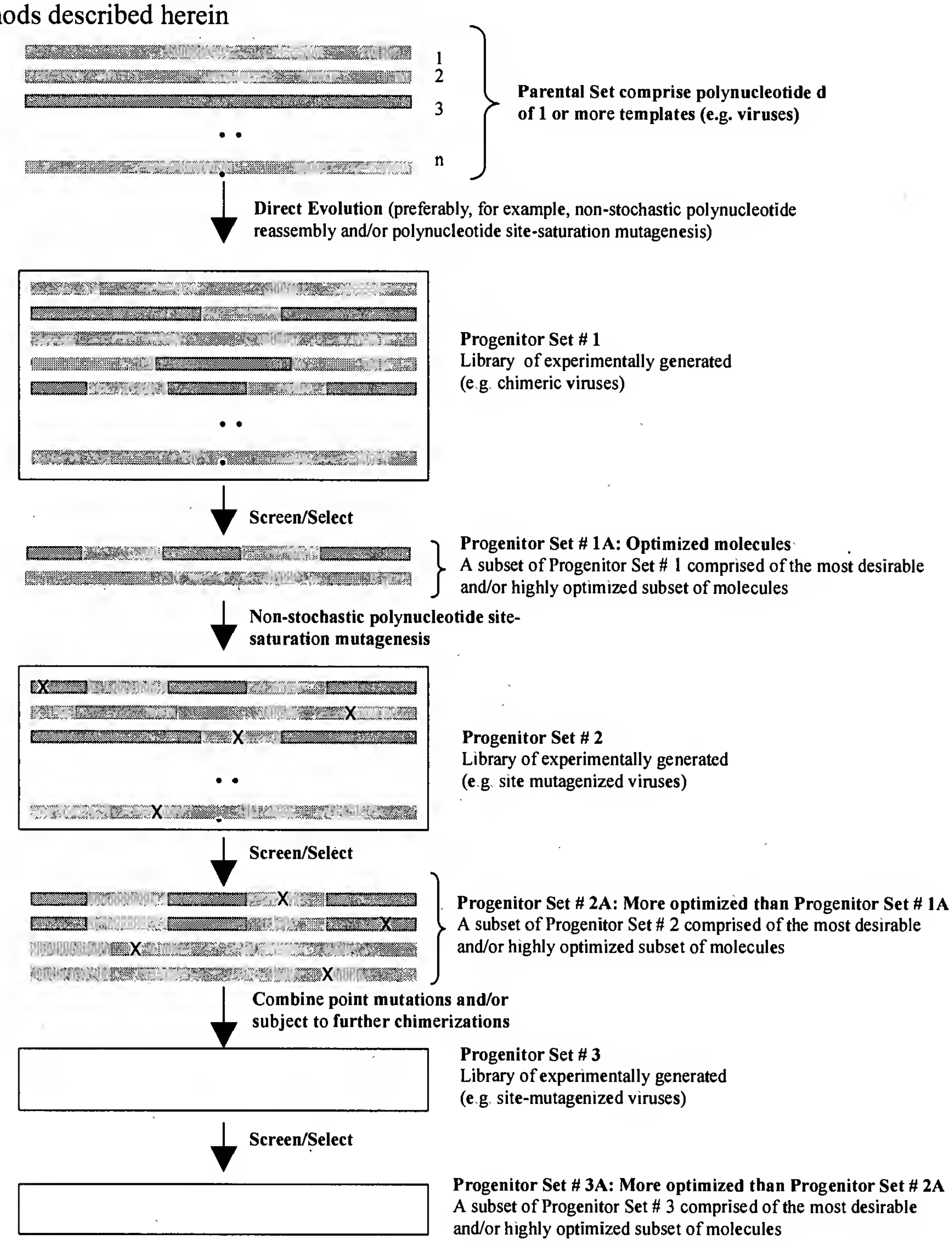


Figure 19A An alignment of two CMV-derived nucleotide sequences from human and primate species.

```
                            1                                                50
AF078102 Rhesus      (1)   ATCGATTTAAACTGCCCGATTGAGGTTGTGGCTGAACAGTTTGTGAGTG-
   M67443 Towne      (1)   --------------------------GCCATGGCATCGGTACTGGGTCC

                            51                                              100
AF078102 Rhesus     (50)   -TTTTGCAAGGTAGATATGACACTGCTTTTCCAGAAGTGCCGCTGAAGCCT
   M67443 Towne     (24)   CATTTGCGGGCACGTGCTGAAAGCCGTGTTTAGTCGCGGCGAC-ACGCCG

                            101                                             150
AF078102 Rhesus     (99)   GGGGTACATATCTGAAGCAGCGAGATGAGCGTCCCGAGGACGTG-ATACG
   M67443 Towne     (73)   GTG--------CTGCCGCA-CGAGACGCGACTCCTGCAGACGGGTATCCA

                            151                                             200
AF078102 Rhesus    (148)   C-TGCCACTACGGC-GTGTTCAGTTTGCGTAATCTCCTGTTTCCAACGTTG
   M67443 Towne    (114)   CGTGCCCGTGAGCCAGCCCTCGCTGATGCTGGTGTCCCAGTACACGCCCG

                            201                                             250
AF078102 Rhesus    (196)   TCT-GAG--TATGTTACAG-GGTGTTTATGTGGCGACAATACGATGTAACCG
   M67443 Towne    (164)   ACTCGACGCCATGCCAGCGCGGCGACAATCAGGTGCAGGTGCAGCACACG

                            251                                             300
AF078102 Rhesus    (242)   TG-TTTACGAGAATATATTAAAAAGAATTCTGGTAGTATCATTCCGTTGC
   M67443 Towne    (214)   TACTTTACGGGCAGCGAGGTGGAGAACGTGTCGGTGAA------CGT-GC

                            301                                             350
AF078102 Rhesus    (291)   GCAACGCCTGTAGGTGGTTTCTCGTCCTTCGCTCTGGTATAGATATTGCC
   M67443 Towne    (257)   ACAACGCCACGGGCCGGA------------GC----------ATCTGCCC

                            351                                             400
AF078102 Rhesus    (341)   CAGGTAGAAACCCGT-TCGTTCGACGAGTAT-CTTTGCTGCAAAGAGAAA
   M67443 Towne    (285)   CAGCCAAGAGCCCATGTCGATCTATGTGTACGGCTGCCGCTCA-AGATG

                            401                                             450
AF078102 Rhesus    (389)   TTGGAAGCAGTGGGAATATTGGGGTGCAGTTGGTGATGGCAGT-GATG
   M67443 Towne    (334)   CTGAACATCGCCAGCATCAACGTGCACCACTACCC-GTCGGCGGCCGAGC

                            451                                             500
AF078102 Rhesus    (438)   GCAAAAAAATCCAGGAGAGTACGTGTGTGGTTCTGCTGGGAAGGTATGGA
   M67443 Towne    (383)   GCAAACACCGACACCTGCGCGTAGCTGACGCTGTGATTC-ACGGGTCGGG

                            501                                             550
AF078102 Rhesus    (488)   TTTGTGTACGTGTATGACTGG---GACAGTGACGG-ACTCTTTCAGATAG
   M67443 Towne    (432)   CAAGCAGATGTGGCAGGCGCGTCTCACGGTCTCGGGACTGGCCTGCACGC

                            551                                             600
AF078102 Rhesus    (534)   GT-ACGTCACTCAAAGAACTCGCGCAGCACGGTTTACTG-ACATGTGAAT
   M67443 Towne    (482)   GTCAGCAGAACCAGTGGAAA-GAGCCGGACG-TCTACTACAGGTCAGCGT

                            601                                             650
AF078102 Rhesus    (582)   CGGTATATGCCATGCGGAAACGCGCTTCTCGACCAGCGAGGCTCGTTTT
   M67443 Towne    (530)   TCGTGTTTG-----CCAGCAAGGACGTGG--CAGTGCGGCAGGTGGTGTG

                            651                                             700
AF078102 Rhesus    (632)   CAAGTCGAGAAATTGTTGTGTCTCGATCCGACAGACGCACGTGGTCTAGC
   M67443 Towne    (573)   CGCG-CACGAGCTGGTT-TG-CTCCATGGAGAACACGCGCGCAACGAAGA
```

Figure 19B

```
                            701                                               750
AF078102 Rhesus    (682)   TAAGACCGCAGAAGAGTTTCATGGCGTTAACGTAGTAGTGAAAACCC-CT
   M67443 Towne    (620)   T--GCAGGTGATAGGTGACCAGTACGTCAAGGTGTACCTGGAGTCCTTCT

                            751                                               800
AF078102 Rhesus    (731)   GGCCGATCGGAGGCCGATCCGTTGCTCCTTCTGG--GTACTGTGGAGAAG
   M67443 Towne    (668)   GCGAGGACGT-GCCCTCCGGCAAGCTCTTTATGCACGTCACGCTGGGCTC

                            801                                               850
AF078102 Rhesus    (779)   CTACGTCAAATGTATCCCTTTGCTAAAATGAGAGCTGATAACTTTAGTAA
   M67443 Towne    (717)   TGACGTGGAAGAGGACCTGACGATGACCCGCAACGCGCAACCCTTCATGC

                            851                                               900
AF078102 Rhesus    (829)   TGTC--ATAAAGTATATTAATCAACGCATGTGTTGCCGA------TGGTA
   M67443 Towne    (767)   GCCCCCACGAGCGCAACGGCTTTACGGTGTTGTGTCCCAAAAATATGATA

                            901                                               950
AF078102 Rhesus    (871)   --CGTAGTTGGTGTTA-CTGGG-CGTTACGCTAATTTTGGAATTTTGCTG
   M67443 Towne    (817)   ATCAAAGCGGGCAAGATCTCGGACATCATGCTGGATGTGGCTTTTACGTC

                            951                                              1000
AF078102 Rhesus    (917)   AGCTGTGGAATAATTCTGTTGGACGAGTGCGGCGTGTGTTATGTGC-TGA
   M67443 Towne    (867)   ACACGAGCATT--TTGGGCTGCT-GTGTCGCAAGAGCATCCCGGGCCTGA

                           1001                                              1050
AF078102 Rhesus    (966)   GAATCGACGAAAGCGACGTGTTT--CGTCTAGCCGATAACATCCAAATGT
   M67443 Towne    (914)   GCATGTCAGGTAACCTATTGATGAACGGGCAGCAGAT--CTTCCTGGAGG

                           1051                                              1100
AF078102 Rhesus   (1014)   TGTTCAGATGCCGCTTTGTTAAGCTCCGTGGCATTACGCGATTTGACCGC
   M67443 Towne    (962)   TGC----AAGCGA--TACGCGAGA-CCGTGGAACTGCGTCAGT--AC---

                           1101                                              1150
AF078102 Rhesus   (1064)   GGATTGCGTGGCGAGGCTCGTCTG----GAGAGTGAGT-GCTACTGCTAT
   M67443 Towne   (1000)   -GATCCCGTGGCTGCGCTCTTCTTTTTCGATATCGACTTGCTGCTGC-AG

                           1151                                              1200
AF078102 Rhesus   (1109)   GACGGTGCCCGTAAGCAAGATCTCTGTTGGTGCTCTATGGTCAACACAGT
   M67443 Towne   (1048)   CGCGGCGCTCAGTACAGCGAAGAGCCCACCTTCAGCA--GCCAGTATCGC

                           1201                                              1250
AF078102 Rhesus   (1159)   TACGGCCGAGCAGCTCTGTGGTGCGT-ACGACTGGCTCACGCGCAGTAAT
   M67443 Towne   (1096)   ATCCA--GGGCAAGCTTCAGTACCGACACACCTGGG--ACCGGCACGACG

                           1251                                              1300
AF078102 Rhesus   (1208)   CGCGCTCATGATAAAATACGTACCAGTTGTTGTTGGCGACGTAGTGATTT
   M67443 Towne   (1142)   ACGGTGCCGCCCAGGGCGACGACGACGTCTGGACCAGCGGATCGGACTCC

                           1301                                              1350
AF078102 Rhesus   (1258)   GATGCCTACCGGAGTATTGCAAGAGAATCAGAATTGGTGCTTTCCGTGGA
   M67443 Towne   (1192)   GACGAGGAACTC-GTAACCACCGAGCGCAAGACGCCCCGCGTTACCGGCG

                           1351                                              1400
AF078102 Rhesus   (1308)   GGAGTGTCACGGTTCTACAAGGGCC-CCAAAGCGGTGCGTGGCAA--GAA
   M67443 Towne   (1241)   GCGGCGCCATGG-----CGGGCGCCTGCACTTGCGCGGCCCGGAAACGCA
```

Figure 19C

```
                                 1401                                              1450
AF078102 Rhesus       (1355)    AATGATGAGTTAACGGAGAAAACGTCTGATGGTACTGTGATGTTTGGTGT
    M67443 Towne      (1286)    AATCAGCA-TCCTCGCCGACGGCGTGCACGGCGGGCGTTATGACACGCCGG

                                 1451                                              1500
AF078102 Rhesus       (1405)    A-GACAGAGGTTTTTCTGGCAGAACCGTAAAGTTCGAGGGTGAAAGCGAT
    M67443 Towne      (1335)    CCGCGTTAAGGCCGAGTGC----ACCGTCGCGCCCGAAGAGCACACCGAC

                                 1501                                              1550
AF078102 Rhesus       (1454)    GACTACACAGAAGAGGAGGAAGATGATGATAACGAGTCAGAAGAAACGCC
    M67443 Towne      (1381)    GAGGATTGCGACAACGA--AATCC----ACAATGCGGCGTGTTCACCTG

                                 1551                                              1600
AF078102 Rhesus       (1504)    TGAGCATCACGCTGTTGTCATTGTTTGAGATGAAACAAACGGCAGTGTTG
    M67443 Towne      (1425)    GCCGCCCTGGCAGGCCGGCATCCTGGCCGCAACCTGGTGCCCATGGTTG

                                 1601                                              1650
AF078102 Rhesus       (1554)    ATTGTTATGATGATGAGAGTGAGTCAT-GTCTG--TCCT-GAGATACCCA
    M67443 Towne      (1475)    CTACGGTTCAGCGTCAGAATCTGAAGTACCAGGAGTTCTTGTGGCACGGC

                                 1651                                              1700
AF078102 Rhesus       (1600)    TGATGCAGCAGCCCGAGAGATTATGGCGATAATCCAAAAGGAATGGCA--
    M67443 Towne      (1525)    --AACGACATGTACGG--CATCTTCGCGGAATTGGAAGGCGTATGGCAGC

                                 1701                                              1750
AF078102 Rhesus       (1648)    ---------AAACGAAGAGCCGGTTAGCAATGAAAGAGAATTGTTAGTGC
    M67443 Towne      (1571)    CCGCTGCGCAACGCAAACGTCGCCGCGACCGGCAAGACGCCT-TGCCGG

                                 1751                                              1800
AF078102 Rhesus       (1689)    GCGCTGCA---GGACGA-GGTAAACATATGAAAAAGTGGCATTGTTGCGT
    M67443 Towne      (1620)    GCCATGCATCGGCTCGACGGCCAAAAAGCACCGAGGTTGAGCCACCGGC

                                 1801                                              1850
AF078102 Rhesus       (1735)    GGAAAGGGTTAGCA------TTTATGACCCGTTGAATGCTAATATTCAGT
    M67443 Towne      (1670)    GCGCACGCTTAGGACGACTCTATAAAAACCGACGTCCAGTCAGACACGGG

                                 1851                                              1900
AF078102 Rhesus       (1779)    GCTTTGATTAAACAATGCATTTGTATTGAATTATCTAATTCGGT--GTA
    M67443 Towne      (1720)    ACTTTTGGCCGCCA---CACCTGTCGCGGCTGCTATATTTGCGACAGTTG

                                 1901                                              1950
AF078102 Rhesus       (1827)    CGGTGTCATTTGTGCATGGAGTGGTGGGAAAGAGATTACATATTTAGACA
    M67443 Towne      (1767)    CGGGAAGCCTTGCCGA--GCTCCCACGAAGAGCCGTT-CACCTTTGCCGCA

                                 1951                                              2000
AF078102 Rhesus       (1877)    -CAGGTATGAGTTGTGAAAGCTGAGTGAAGATCATGCAATTGTGGGAGGA
    M67443 Towne      (1814)    TGCGCTGAGCCCCCCC----GTCATCGCGGCTTCGCGATGTCTCAGGCAT

                                 2001                                              2050
AF078102 Rhesus       (1926)    GGTCCGGGTCGTTCGTTCTGTGTTTCT-GTTACGAGTTTAGATTTTATTA
    M67443 Towne      (1860)    CGTCC---TCGCCCGGTGAGGCACCGCTCGTCGGAAGCGGCGGCGATCAGC

                                 2051                      2077
AF078102 Rhesus       (1975)    TGGTTGAAAGTGTTGTATTTCTAA---
    M67443 Towne      (1907)    GAGGCGGAAGCGGCG-AGCGGAAGCTT
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Figure 20: An alignment of the IFN-gamma nucleotide sequences from human, cat, rodent species.

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                                               1                                                50
AF081502 Marmota monax IFN-gamma       (1)     --------------------GGCCTAACTCTGTCTGAAACGATGAAATAG
   D30619 Felis catus IFN-gamma        (1)     CTACTGATTTCAACTTCTTTGGCCTAACTCTG--CGAAACGATGAATTAG
    X87308 Homo sapien IFN-gamma       (1)     --------------------------------------------------

                                               51                                              100
AF081502 Marmota monax IFN-gamma      (31)     ACAAGTTATTTCTTGGCTTTTCAGCTCTGCATCATTTTGGGTTCTTCTAG
   D30619 Felis catus IFN-gamma       (49)     ACAAGTTTTATTTTCGCTTTCCAGCTTTGCATAATTTTGTGTTCTTCTGG
    X87308 Homo sapien IFN-gamma       (1)     --------------------------------------------------

                                               101                                             150
AF081502 Marmota monax IFN-gamma      (81)     CTGTTACTCCCAGGACACAGTTAATAAAGAAATAGAAGATTTAAAAGGAT
   D30619 Felis catus IFN-gamma       (99)     TTATTACTGTCAGGCCATGTTTTTTAAAGAAATAGAAGAGCTAAAGGGAT
    X87308 Homo sapien IFN-gamma       (1)     -TGTTACTGCCAGGACCCATATGTAAAAGAAGCAGAAAACCTTAAGAAAT

                                               151                                             200
AF081502 Marmota monax IFN-gamma     (131)     ATTTTAATGCAAGTAATTCAAATGTATCAGATGGCCGGGTCTCTCTTCTTG
   D30619 Felis catus IFN-gamma      (149)     ATTTTAATGCAAGTAATCCAGATGTAGCAGATGGTGGGTCGCTTTTCGTA
    X87308 Homo sapien IFN-gamma      (50)     ATTTTAATGCAGGTCATTCAGATGTAGCGGATAATGGAACTCTTTTCTTA

                                               201                                             250
AF081502 Marmota monax IFN-gamma     (181)     GATATTTTGGATAAATGGAAAGAGGACAGTGACAAAAAAGTAATCCAGAG
   D30619 Felis catus IFN-gamma      (199)     GACATTTTGAAGAACTGGAAAGAGGAGAGTGATAAAACAATAATTCAAAG
    X87308 Homo sapien IFN-gamma     (100)     GGCATTTTGAAGAATTGGAAAGAGGAGAGTGACAGAAAAATAATGCAGAG

                                               251                                             300
AF081502 Marmota monax IFN-gamma     (231)     CCAAATTGTCTCTTTCTACTTCAAAGTCTTTGAACACTTAAAAGACAA--
   D30619 Felis catus IFN-gamma      (249)     CCAAATTGTCTCCTTCTACCTGAAAATGTTTGAAAACCTGAAAGATGATG
    X87308 Homo sapien IFN-gamma     (150)     CCAAATTGTCTCCTTTTACTTCAAACTTTTTAGAAACTTTAAAGATGA--

                                               301                                             350
AF081502 Marmota monax IFN-gamma     (279)     -CAAGATCATCCAAAGGAGCATGGACACCATCAAGGGGGATCTTTTTGCT
   D30619 Felis catus IFN-gamma      (299)     ACCAGCGCATTCAAAGCAGCATGGACACCATCAAGGAAGACATGCTTGAT
    X87308 Homo sapien IFN-gamma     (198)     -CCAGAGCATCCAAAAGAGTGTGGAGACCATCAAGGAAGACATGAATGTC

                                               351                                             400
AF081502 Marmota monax IFN-gamma     (328)     AAGTTCTTCAACAGCAGTACCAATAAGCTGCAGGACTTCCTAAAGGTCTC
   D30619 Felis catus IFN-gamma      (349)     AAGTTGTTAAATACCAGCTCCAGTAAACGGGATGACTTCCTCAAGCTGAT
    X87308 Homo sapien IFN-gamma     (247)     AAGTTTTTCAATAGCAACAAAAAGAAACGAGATGACTTCGAAAAGCTGAC

                                               401                                             450
AF081502 Marmota monax IFN-gamma     (378)     TCAAGTTCAGGTAAATCACCTCAAGATCCAGCCTAAAGCAGTGAGTCAAC
   D30619 Felis catus IFN-gamma      (399)     TCAAATCCGTGTGAATGATCTGCAGGTCCAGCGCAAAGCAATAAATGAAC
    X87308 Homo sapien IFN-gamma     (297)     TAATTATTCGGTAACTGACTTGAATGTCCAACGCAAAGCAATACATGAAC

                                               451                                             500
AF081502 Marmota monax IFN-gamma     (428)     TCAAGAAAGTGATGAATGATCTGTTACCACACTCTACCCTAAGGAAGCCA
   D30619 Felis catus IFN-gamma      (449)     TCTTCAAAGTGATGAATGATCTCTCACCAAGATCTAACCTGAGGAAGCCG
    X87308 Homo sapien IFN-gamma     (347)     TCATCCAAGTGATGGCTGAACTGTCGCCAGCAGCTAAAACAGGGAAGCCG

                                               501                                             550
AF081502 Marmota monax IFN-gamma     (478)     AAAAGGAGTCAGTCTTCGATTCGGGGTCGGAGAGCATCCAAATAACAGTC
   D30619 Felis catus IFN-gamma      (499)     AAAAGGAGCCAGAATCTGTTTCGAGGCCGTAGAGCATCGAAATAATGGTT
    X87308 Homo sapien IFN-gamma     (397)     AAAAGGAGTCAGATGCTGTTTCGAGGTCGAAGAGCATCCAG---------

                                               551             569
AF081502 Marmota monax IFN-gamma     (528)     CTCATGCCTGC--------
   D30619 Felis catus IFN-gamma      (549)     GTCCTGCCTGCAATATTTG
    X87308 Homo sapien IFN-gamma     (439)     -------------------
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